Isolation and structure determination of new siderophore albachelin from *Amycolatopsis alba*

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Title: Isolation and structure determination of new siderophore albachelin from *Amycolatopsis alba*

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18
19 **Abstract**

20 A new siderophore named albachelin was isolated from iron deficient culture of
21 *Amycolatopsis alba*. The planar structure of albachelin was elucidated by the combination of
22 ESI-MS/MS experiment and NMR spectroscopic analyses of the gallium (III) complex. The
23 structure of albachelin was determined to be a linear peptide consisting of 6 mole of amino acids
24 including 3 mole of serine, one mol each of *N*- α -acethyl-*N*- δ -hydroxy-*N*- δ -formylornithine, *N*- α -
25 methyl-*N*- δ -hydroxyornithine, and cyclic *N*-hydroxyornithine. The stereochemistries of amino
26 acids constituting albachelin were analyzed by applying modified Marfey method to the
27 hydrolysate of albachelin. Based on bioinformatics, we deduced and discussed the possible
28 biosynthetic gene cluster involved in albachelin biosynthesis from the genome sequence of *A.*
29 *alba*. By prediction of substrates for adenylation domains, a non-ribosomal peptide
30 biosynthetase gene (AMYAL_RS0130210) was proposed to be the main biosynthetic gene for
31 albachelin biosynthesis. The related genes including transporter for siderophore were found near
32 the NRPS gene as a gene cluster.

33
34 Keywords: siderophore, *Amycolatopsis alba*, peptide, biosynthesis

36

37 **Introduction**

38 Recent genome sequencing of actinomycetes has now indicated that the genome of each strain
39 contains multiple biosynthetic gene clusters which are predicted to encode the assembly-line
40 biosynthesis of polyketides or nonribosomal peptides (Clardy 2006; Komaki et al. 2014; Komaki
41 et al. 2012; Zerikly and Challis 2009). According to development of bioinformatics and
42 accumulation of biosynthetic gene data, the structural prediction of natural products has been
43 possible from biosynthetic genes predicted by the genome-mining (Bachmann and Ravel 2009;
44 Rottig et al. 2011). However under normal culture conditions, many of these gene clusters are
45 not expressed enough to produce the resulting compounds (Ochi and Hosaka 2013), and
46 therefore it is claimed that genome resources of actinomycetes remain unexploited although
47 secondary metabolite biosynthetic genes exist abundantly in their genomes and possess a wide
48 diversity.

49 In iron deficient condition, some bacteria secrete siderophores which are defined as small
50 molecular weight compounds with high-affinity of iron chelating (Ahmed and Holmstrom 2014).
51 According to the biosynthetic system, siderophores have been classified into two groups
52 including one NRPS (Non Ribosomal Peptide Synthesis) dependent (Crosa and Walsh 2002)
53 such as ferrichrome (Mercier and Labbe 2010) and the other NRPS independent (Challis 2005)
54 such as desferrioxamine (Barona-Gomez et al. 2004). The NRPS protein is mostly a large
55 molecular multi-enzyme which functions as the assembly-line to produce the resulting peptide
56 through a series of amino acid building modules lined up in parallel (Sieber and Marahiel 2005).
57 Each module in NRPS causes successive elongation of the peptide by one amino acid with
58 enzymatic function of condensation (C) domain. Each module normally contains a basic set of

three domains, an adenylation (A) domain for the recognition of specific amino acid substrate, a peptidyl carrier protein (PCP) that binds the growing peptide chain and the incoming aminoacyl unit, and a C domain to catalyze peptide bond formation between amino acids on neighboring PCPs. Optional domains such as epimerization (E) and *N*-methylation (MT) domains sometimes exist on the modules and modify amino acid to afford unnatural amino acid such as D-amino acid and *N*-methyl amino acid. A thioesterase (TE) domain normally exists at the end of assembly-line of NRPS to release full-length of the peptide.

The A domain of NRPS in siderophore biosynthesis has been indicated to have high specificity to amino acid substrate (Challis et al. 2000). Therefore structural prediction of siderophores has comparatively been accomplished rather than other classes of NRPS biosynthesized peptides. A siderophore coelichelin was the first example of structure-predicated peptide (Challis and Ravel 2000), and the structure elucidation of coelichelin revealed an unique NRPS system in which one module is reused and others are skipped (Lautru et al. 2005). Erythrochelin was also isolated and structure-elucidated based on genome mining of *Saccharopolyspora erythraea* (Robbel et al. 2010), and the function of NRPS was determined by gene disruption (Lazos et al. 2010). A new siderophore biosynthesized by NRPS was found and isolated from *Streptomyces peucetius* based on genome mining (Park et al. 2013). Recently, we isolated and structure-elucidated siderophore scabchelin from plant-pathogen *S. scabies*, and found the biosynthetic gene cluster in its genome (Kodani et al. 2013a). An unusual mixed-ligand siderophore amychelin was isolated from *Amycolatopsis* sp. AA4 and its biosynthetic gene cluster was determined by whole genome sequencing (Seyedsayamdost et al. 2011). Above all, isolation and structural elucidation of new siderophores from bacteria whose genome sequence was determined gave the new insights of biosynthetic genes by applying bioinformatics. On the basis of these circumstances, we isolated

a new siderophore named albachelin from type strain of *Amycolatopsis alba*, and the structure was determined by the interpretation of NMR and MS spectra. Using bioinformatics with the whole genome data of *A. alba*, the possible biosynthetic genes were deduced and discussed.

Results

In the course of screening for siderophore production, we found that *Amycolatopsis alba* (type strain, JCM 10030) produced a siderophore on iron-deficient culture condition. Following the method in previous paper (Kodani et al. 2013b), cultivation of *A. alba* was performed using 2L of iron deficient media. To avoid the contamination of ferric ion, flasks and funnels made of polystyrene were used to culture and harvest. After cultivation for 7 days, the bacterial cells were removed from the culture media by filtration. 1M FeCl₃ solution (1 mL) was added to the spent culture media to generate the complex of siderophore with ferric ion. The culture media was concentrated to the aqueous solution up to 50 mL by rotary evaporator. The concentrated material was subjected to open column chromatography using hydrophobic resin CHP-20P with elution of 10% MeOH, 60% MeOH, and MeOH. The 60% MeOH fraction was subjected to HPLC purification to yield 8.2 mg of the ferri-albachelin (Fe-albachelin). The measurement of ESI-TOF mass spectrum of ferri-albachelin gave an ion peak at m/z 789.3. Since the presence of ferric ion is not compatible with the NMR spectroscopy analysis, the conversion of the ferric siderophore into gallium ion complex via desferri-albachelin was performed following previous report (Kodani et al. 2013a; Kodani et al. 2013b). As a result, desferri-albachelin (**1** in Fig. 1) and gallium-complex of albachelin (Ga-albachelin) were obtained with the yield of 1.0 and 4.0 mg, respectively. After HPLC purification, the presence of Ga-albachelin was determined by ESI-TOF MS analysis, which gave ion peaks at m/z 802.6 and 804.6 with intensity ratio of 6:4, which indicated gallium complex.

Desferri-albachlin (**1**) was isolated as a white powder after lyophilization, and the molecular formula of **1** was determined to be C₂₈H₄₉N₉O₁₄ by FT-ICR ESI-MS analysis (m/z calculated for C₂₈H₅₀N₉O₁₄⁺: 736.3471 found: 736.3476 for [M+H]⁺). The analyses of NMR spectra including ¹H, ¹³C, DEPT-135, DQF-COSY, TOCSY, NOESY, HMBC, HSQC, and ¹H-¹⁵N HSQC were performed on Ga-albachelin dissolved in 0.5 mL of DMSO-*d*₆. The ¹H NMR spectral data showed peptidic characteristic with peaks of several amide residues over 8-9 ppm and α-protons over 4-5 ppm. By the interpretation of DQF-COSY and TOCSY, proton spin system of each amino acid was constructed as shown by bold line in Fig. 2a. The assignments of C-H spin system were performed by the interpretation of HSQC data (Table 1), and revealed that albachelin consisted of 6 mole of amino acids including 3 mole of Ser and one mol each of N-α-acethyl-*N*-δ-hydroxy-*N*-δ-formylornithine (*N*-Ac hfOrn), *N*-α-methyl-*N*-δ-hydroxyornithine (*N*-Me hOrn), and cyclic *N*-hydroxyornithine (chOrn). The existence of an acetyl residue was confirmed by the HMBC correlation from methyl protons (1.83 ppm) to carbonyl carbon (169.1 ppm). The HMBC correlation from amide proton (7.62 ppm) to the same carbonyl carbon (169.1 ppm) indicated that acetyl residue attached to amide proton of *N*-Ac hfOrn1. The presence of formyl residue was indicated by HSQC correlation from singlet proton (7.99 ppm) to characteristic chemical shift value of 152.2 ppm. The formyl residue was indicated to be attached to δ position in *N*-Ac hfOrn, by the HMBC correlation from δ-protons (3.29 and 3.49 ppm) to carbonyl carbon (152.2 ppm). As shown in Fig. 2a by one end arrow, the HMBC correlations from α-proton or amide proton to carboxyl carbon were used to establish the connections between *N*-Ac hfOrn1/Ser2, Ser4/Ser5, and Ser5/chOrn6. As shown in Fig. 2a by double end arrow, the NOESY correlation between α-proton of Ser2 and δ-protons of *N*-Me hOrn3 indicated the connection between Ser2 and *N*-Me hOrn3. The NOESY correlation

between α -proton of *N*-Me hOrn3 and amide proton of Ser4 indicated the connection between *N*-Me hOrn3 and Ser4. The hydroxyl residues in *N*-Ac hfOrn1, *N*-Me hOrn3 and chOrn6 were not detected by ^1H NMR, however the result of ESI-MS/MS experiment supported the positions of hydroxyl residues indicated in Fig. 2b. In ESI-MS/MS analyses of albachelin, the *b*-series of product ions at m/z 606 ($[\text{M}+\text{H}-130]^+$), 519 ($[\text{M}+\text{H}-130-87]^+$), 432 ($[\text{M}+\text{H}-130-87-87]^+$), 288 ($[\text{M}+\text{H}-130-87-87-144]^+$), and 201 ($[\text{M}+\text{H}-130-87-87-144-87]^+$) were observed. The observed neutral losses of 130, 87, 144 correspond to those expected for chOrn, serine, and *N*-Me hOrn, respectively, further corroborating the results of the NMR spectroscopic analyses. The *y*-series product ion peaks were observed at m/z 536, 449, 305, and 208, which also confirmed the sequence of the peptide as shown in Fig. 2b. Above all, the structure of albachelin was determined as **1** in Fig. 1.

To elucidate the absolute stereochemistries of amino acids, the hydrolysate of ferri-albachelin was derivatized with *N* α -(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA), and the derivative was subjected to HPLC analysis to compare with the standard amino acid derivatives with L-FDLA or D-FDLA (Harada et al. 1996). To obtain Orn and *N*-Me Orn, hydrogen iodide (HI) was used for hydrolysis with reduction. Regarding HPLC analysis for stereochemistries of Ser residues, 2 mole of L-Ser and 1 mol of D-Ser were detected. Although 2 mole of L-Ser and 1 mol of D-Ser were indicated to be contained in the molecule, the specific positions of L- and D-Ser in the molecule were not determined. As for Orn, 1 mol each of L- and D-Orn was detected, however the specific positions of L- and D-Orn in the molecule were also not determined. The remaining amino acid, *N*-Me Orn was determined to be L-form, since only L-*N*-Me Orn was detected by HPLC analysis.

Discussion

151 We searched the albachelin biosynthetic gene cluster in the complete genome sequence of *A.*
152 *alba* type strain (DSM 44262) recently released in public (GenBank accession number
153 ARAF00000000.1), and found an NRPS gene cluster shown in Fig. 3a and Table S1. Most of the
154 proteins encoded in the cluster are homologous to those in gene clusters for erythrochelin,
155 coelichelin and scabichelin, which are siderophores structurally related to albachelin (Table S1).
156 The large NRPS protein (7406 amino acids, AMYAL_RS0130210) comprised 6 modules
157 composed of 21 domains, each module containing a set of C, A, and PCP domains essential for
158 NRPS modules (Fig. 3b). This suggested the NRPS product to be a hexapeptide. To predict
159 amino-acid substrates recognized by A domains in the NRPS, we compared the substrate
160 specificity-determining residues with those of NRPSs for coelichelin, erythrochelin and
161 scabichelin synthesis. Consequently, A domain of module 1 was predicted to recognize hfOrn
162 based on the similarities (Table 2). A domains of modules 2, 4 and 5, and those of modules 3
163 and 6 were indicated to recognize Ser and hOrn, respectively. As optional domains, module 3
164 contained an MT domain, suggesting that hOrn3 within the hexapeptide is *N*-methylated. In
165 addition, modules 2 and 6 of the NRPS contained an E domain each, suggesting that absolute
166 stereochemistry of the Ser2 and hOrn6 is D-form. These predicted amino-acid building blocks
167 completely corresponded to those of albachelin. Upstream of the NRPS, two genes encoding
168 lysine/ornithine *N*-monooxygenases and formyltransferase were present (AMYAL_RS0130205
169 and AMYAL_RS0130200), which are essential for biosynthesis of L-hOrn and L-hfOrn,
170 respectively. Downstream of the NRPS, AMYAL_RS0130220 and AMYAL_RS0130225 to
171 AMYAL_RS0130245 encoded MbtH-like protein and proteins for ferric-siderophore
172 export/uptake, respectively (Table S1). These results strongly suggested this gene cluster to be
173 responsible for albachelin synthesis.

174 Regarding biosynthesis of unusual amino acids including L-hOrn and L-hfOrn, a flavin-
175 dependent monooxygenase encoded by AMYAL_RS0130205 initially catalyzes *N*5-
176 hydroxylation of L-Orn (Fig. 3b). The L-hOrn molecules are not only directly recognized by A
177 domains of modules 3 and 6 as the building blocks, but also undergoes *N*5-formylation catalyzed
178 by the formyltransferase encoded by AMYAL_RS0130200 yielding L-hfOrn, as reported in the
179 biosynthesis of coelichelin (Lautru et al. 2005) and scabichelin (Kodani et al. 2013a). Each
180 amino-acid building block (L-hfOrn for module 1, L-hOrn for modules 3 and 6, Ser for modules
181 2, 4, 5) is converted to aminoacyl adenylate by each the A domain, and transferred on to the
182 adjacent PCP domain within each module to form the corresponding aminoacyl thioesters. When
183 L-hfOrn is loaded onto module 1, the C domain catalyzes acetylation yielding *N*-Ac hfOrn
184 according to the mechanism reported in erythrochelin synthesis (Robbel et al. 2010). L-hOrn
185 loaded onto module 3 undergoes *N*-methylation by the MT domain. The L-Ser residue of module
186 2 and L-hOrn residue of module 6 undergo α -carbon epimerization catalyzed by the E domain
187 within each module, and then C domains of modules from 2 to 6 catalyze five successive *N*-
188 acylation reactions to yield a L, D, L, L, L, D - hexapeptidyl thioester attached to the PCP
189 domain of module 6. The stereochemistry of three Ser residues in albachelin was expected to be
190 D-form at Ser2 and L-forms at Ser4 and Ser5, although specific positions of two L- and one D-
191 Ser residues in albachelin were not determined in the present study. However we detected L-Ser
192 and D-Ser from hydrolysate of albachelin at the ratio of 2:1 by modified Merfey method, which
193 corresponds to the speculation. In the same way, we detected L-Orn and D-Orn at the ratio of
194 1:1, which also seems to be reasonable, considering that *N*-Ac Orn1 and chOrn6 should
195 biosynthetically be L- and D-forms. The remaining amino acid, *N*-Me Orn3 was determined to
196 be L-form, which also agrees with the speculation. Interestingly, the NRPS of albachelin lacks

C-terminal TE domain as same as that of scabchelin (Kodani et al. 2013a). In the case of biosynthesis of structurally related siderophore amychelin which has chOrn residue at C-terminal, a putative standalone α,β -hydrolase (AmcB) was proposed to get involved in final release of peptide. (Seyedsayamdost et al. 2011) In *A. alba* genome, AMYAL_RS0125485 (WP_020634094) near the albachelin biosynthetic gene cluster encode a protein with 48% sequence identity (61% sequence similarity) to AmcB, suggesting likely to be an α,β -hydrolase. Hence, the putative α,β -hydrolase may catalyze final peptide chain release. Above all, we concluded that the NRPS gene cluster (Figure 3a) including main biosynthetic NRPS gene (AMYAL_RS0130210) was reasonably be the gene cluster of albachelin biosynthesis.

Materials and Methods

Bacterial strain and culture condition

Amycolatopsis alba JCM 10030 (type strain) was obtained from the Japan Collection of Microorganisms (JCM) Microbe Division at the RIKEN BioResource Center. The iron deficient medium was prepared by adding 2g of K₂SO₄, 3g of K₂HPO₄, 1g of NaCl, 5g of NH₄Cl in 1 L of deionized water. To remove ferric ions, the solution (1 L) was stirred with 50g of weakly acidic cation exchange resin Chelex-100 sodium form (Bio-rad, CA, USA) for 2 h. The solution was filtrated with paper filter (Whatman No.1, GE Healthcare Life Sciences, Buckinghamshire, England) and added with 80 mg of MgSO₄, followed by autoclaving. The separately sterilized solutions (10 mL each) of CaCl₂•H₂O (10 mg/mL), glucose (250 mg/mL), and 0.5% yeast extract (Difco) were added to 1L of sterile medium in clean bench. *A. alba* was cultured by total 2 L of iron-deficient media with incubated rotary shaker (100 rpm, 30 °C) for 7 days.

Isolation of albachelin

The culture medium of *A. alba* was harvested by filtering with paper filter to remove bacterial cells (Whatman No.1). The medium was added with 0.5 mL of 1M FeCl₃ and evaporated using rotary evaporator to concentrate to 50 mL of the final volume. The concentrated solution was subjected to open column chromatography with hydrophobic resin CHP20P (Mitsubishi Chemical, Tokyo, Japan) eluted with 10% MeOH, 60% MeOH, and MeOH. The 60%MeOH fraction was concentrated and repeatedly subjected to HPLC purification to obtain 8.2 mg of ferri-albachelin using C18 column (4.6× 250 mm, Wakopak Handy-ODS, WAKO, Osaka, Japan), eluted with 2% MeCN/98%water containing 0.05%TFA at a flow rate of 1 mL/min and monitored at UV-VIS absorbance 435 nm.

Conversion of ferri-albachelin into Ga-albachelin via desferri-albachelin

Ferri-albabachelin (6.0 mg) was dissolved in 3 mL of water. The solution was mixed with 3 mL of 1M 8-quinolinol and stirred at room temperature for 30 min. Total 2 times of the two layer partition were performed using 6 mL of CH₂Cl₂ each time to get rid of ferri-8 quinolinol. The water layer was immediately collected and lyophilized by freeze-dryer. After dissolving the dry material in 2 mL of water, HPLC purification was performed using C18 column (4.6 × 250 mm, Wakopak Handy-ODS), eluted with 4% MeCN/96%water containing 0.05%TFA at a flow rate of 1 mL/min, and monitored at UV-VIS absorbance 215 nm to yield 6.0 mg of desferri-albachelin. Desferri-albachelin (5.0 mg) was dissolved in 2 mL of distilled water, and 10 mg of Gallium chloride (Sigma Aldrich, MO, USA) was added to convert it to Ga-albachelin. After HPLC purification in the same condition as described above, 4.0 mg of Ga-albachelin was obtained.

NMR experiments

A NMR sample was prepared by dissolving the purified peptide in 500 μ l of DMSO- d_6 (Sigma Aldrich). 1D ^1H , ^{13}C , DEPT-135, and all 2D NMR spectra were obtained on Bruker Avance800 spectrometer with quadrature detection (Bruker BioSpin, MA, USA). The 1D ^1H , ^{13}C , DEPT-135 spectra were recorded at 25°C with 15 ppm for proton and 240 ppm for carbon. The following 2D ^1H -NMR spectra were recorded at 25°C with 10 ppm or 15 ppm spectral widths in t_1 and t_2 dimensions in the phase-sensitive mode by States-TPPI method: two-dimensional (2D) double quantum filtered correlated spectroscopy (DQF-COSY), recorded with 512 and 2048 complex points in t_1 and t_2 dimensions; 2D homonuclear total correlated spectroscopy (TOCSY) with MLEV-17 mixing sequence, recorded with mixing time of 80 ms, 256 and 1024 complex points in t_1 and t_2 dimensions; 2D nuclear Overhauser effect spectroscopy (NOESY), recorded with mixing time of 200 ms, 512 and 2048 complex points in t_1 and t_2 dimensions. Water suppression was performed using presaturation method. 2D ^1H - ^{13}C heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond connectivity (HMBC) spectra were acquired at 25°C in the echo-antiecho mode or in the absolute mode, respectively. The ^1H - ^{13}C HSQC and HMBC spectra were recorded with 1024×512 complex points for 12 ppm in the ^1H dimension and 170 ppm in the ^{13}C dimension or for 10 ppm in the ^1H dimension and 220 ppm in the ^{13}C dimension, respectively, at a natural isotope abundance. 2D ^1H - ^{15}N HSQC spectrum was also recorded at 25°C with 1024×64 complex points for 12 ppm in the ^1H dimension and 50 ppm in the ^{15}N dimension in the phase-sensitive mode by States-TPPI method at natural isotope abundance. All NMR spectra were processed using XWINNMR (Bruker). Before Fourier transformation, the shifted sinebell window function was applied to t_1 and t_2 dimensions except for the HMBC spectrum. All ^1H and ^{13}C dimensions were referenced to DMSO- d_6 at 25 °C.

MS experiments

All mass spectra were recorded in the positive-ion mode. ESI-TOF MS spectra of Ferri-albachelin and Ga-albachelin were recorded using a JEOL JMS-T100LP mass spectrometer (JEOL Ltd., Tokyo, Japan). The accurate mass of desferri-albachelin was analyzed using an ESI Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer (Apex II 70e, Bruker Daltonics, MA, USA) and the MS/MS spectrum was recorded on a MALDI-TOF mass spectrometer (4800 plus TOF/TOF Analyzer, AB SCIEX, CA, USA) using α -cyano-4-hydroxy cinnamic acid (Bruker Daltonics) as the matrix. The mass spectrometers were tuned and calibrated using commercially available standard compounds such as “YOKUDELNA” (JEOL, Tokyo, Japan) for the FT-ICR mass analysis and a “Peptide Calibration Standard II” (Bruker Daltonics) for MALDI-TOF mass analyses prior to the measurement.

Modified Marfey method

Ferri-albachelin (1.0 mg) was subjected to acid hydrolysis at 105 °C for 16 h with concentrated HI (0.5 mL, WAKO), and the hydrolysate was dried by freeze-dryer and resuspended in H₂O (200 μ L). To the hydrolysate, 10 μ L of a solution of *N* α -(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA, Sigma-Aldrich) or D-FDLA (Sigma-Aldrich) in acetone was added at the concentration of 10 mg/ml and 100 μ L of 1 M NaHCO₃, after which the mixtures were heated to 80 °C for 3 min. The reaction mixtures were cooled, neutralized with 2 N HCl (50 μ L), and diluted with MeCN (200 μ L). About 20 μ L of each solution of FDLA derivatives was subjected to HPLC analysis with C18 column (Wakopak Handy-ODS, WAKO, 4.6 $\times$ 250 mm). The DAD detector (MD-2018, JASCO, Tokyo, Japan) was used for detection of the amino acid derivatives accumulating the data of the absorbance from 220nm to 420 nm. The HPLC analysis for Ser was performed at a flow rate of 1 mL/min using solvent A (distilled water containing 0.05% TFA) and solvent B (MeCN containing 0.05%TFA) with a linear

gradient mode from 0 min to 60 min, increasing percentage of solvent B from 25% to 35% . The retention times (min) of L- or D-FDLA derivatized amino acids in this HPLC condition were following; L-Ser-L-FDLA (44.6 min), L-Ser-D-FDLA (46.2 min). The HPLC analysis for Orn and *N*-Me Orn was performed at a flow rate of 1 mL/min using solvent A (distilled water containing 0.05% TFA) and solvent B (MeCN containing 0.05%TFA). The isocratic mode was applied for first 20 min with elution of 27% of solvent B, and for next 20min, a linear gradient mode was applied, increasing percentage of solvent B from 27% to 30% . The retention times (min) of L- or D-FDLA derivatized amino acids in this HPLC condition were following; L-Orn-L-FDLA (34.4 min), L-Orn-D-FDLA (17.5 min), L-*N*-Me Orn-L-FDLA (31.5 min), L-*N*-Me Orn-D-FDLA (29.9 min).

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Conflict of interest

The authors had no conflict of interest in undertaking this project.

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374 Figure legends

375 **Fig. 1** Chemical structure of desferri-albachelin (**1**)

376 **Fig. 2** a) Key TOCSY, NOESY, and HMBC correlations of **1**, b) ESI-MS/MS analyses of **1**

377 **Fig. 3** Putative albachelin biosynthetic gene cluster and proposed pathway for albachelin
378 synthesis. (a) Organization of the albachelin biosynthesis gene cluster in *A. alba*. The proposed
379 functions of the protein encoded by the gene cluster are summarized in Table S1. (b) Proposed
380 role of enzymes encoded by AMYAL_RS0130200 to AMYAL_RS0130210 in the biosynthesis
381 of albachelin. Capital letters A, C, E, and MT represent adenylation, condensation, epimerization,
382 and methylation domains. Black filled circle represents a peptidyl carrier protein (PCP) domain.
383

384 Figure 1

385

386 Figure 2

387

388 Figure 3

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a

390

b

391 Table 1. NMR chemical shift values of Ga-albachelin in DMSO-*d*₆

Residue	Position	δH ($J = \text{Hz}$)	δC
<i>N</i> -Ac hfOrn1	<i>N</i> -Ac-CH ₃	1.83 (s)	22.2
	<i>N</i> -Ac-CO		169.1
	NH	7.62 (d, 7.5)	
	CO		170.9
	α	4.43 (m)	50.7
	β	1.39 (m)	27.5
		1.52 (m)	
	γ	1.39 (m)	18.1
		1.50 (m)	
	δ	3.29 (m)	50.1
Ser2		3.49 (m)	
	formyl	7.99 (s)	152.2
	NH	8.35 (m)	
	CO		161.5
	α	4.84 (td, 9.1, 6.1)	48.1
<i>N</i> -Me hOrn3	β	3.46 (m)	60.4
		3.56 (m)	
	<i>N</i> -CH ₃	2.51 (m)	30.7
	NH	8.85 (br)	
	CO		166.8
	α	3.91 (br)	59.2
	β	1.78 (m)	26.6
		1.89 (m)	
	γ	1.73 (m)	22.3
Ser4		1.79 (m)	
	δ	3.53 (m)	49.5
		4.81 (m)	
	NH	8.67 (d, 8.4)	
	CO		169.0
Ser5	α	4.64 (m)	54.6
	β	3.51 (m)	61.8
		3.54 (m)	
	NH	8.36 (m)	
chOrn6	CO		169.8
	α	4.41 (m)	53.8
	β	3.42 (m)	60.8
		3.58 (m)	
	NH	8.34 (d, 8.4)	
	CO		159.5
chOrn6	α	4.62 (m)	44.9
	β	1.66 (m)	26.4
		1.96 (m)	
	γ	1.81 (m)	18.4
		2.12 (m)	
	δ	3.58 (m)	49.5

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